IL-13 (human), ELISA kit

Catalog # **ADI-900-208**

96 Well Enzyme Immunoassay Kit
For use with serum, plasma, and cell supernatants

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

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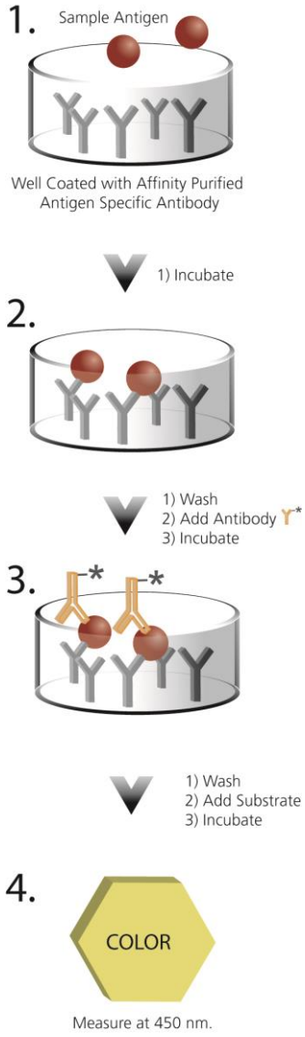


Introduction

The Enzo Life Sciences IL-13 Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determination of IL-13 in plasma, serum and cell supernatants. Please read the complete kit insert before performing this assay.

IL-13 is primarily produced by activated T cells and has many of the same biological properties as IL-4 [1,2]. IL-13 suppresses the expression of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and IL-8 by monocytes/macrophages, induces CD23 expression on B cells and promotes B cell proliferation and stimulates secretion of IgE and IgG_4 and induces IFN-γ production by NK cells [1,2]. IL-13 has been shown to inhibit the replication of HIV both *in vitro* and *in vivo* [3]. IL-13 induces many phenotypes of allergic lung diseases, including airway hyper responsiveness, goblet cell metaplasia and mucus hypersecretion that contribute to airway obstruction [4].

Principle

1. Samples and standards are added to wells coated with a monoclonal antibody to IL-13. The plate is then incubated.
2. The plate is washed, leaving only bound IL-13 on the plate. A yellow solution of horseradish peroxidase conjugated monoclonal antibody to IL-13 is then added. This binds to the IL-13 captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess HRP conjugated antibody. TMB Substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
4. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of IL-13 in the sample.

Materials Supplied

1. **Assay Buffer 13**
   50 mL, Catalog No. 80-1500
   Tris buffered saline containing BSA and detergents

2. **IL-13 Standard**
   Lyophilized, Catalog No. 80-2387
   2 vials containing 300 pg/vial of recombinant IL-13

3. **IL-13 Clear Microtiter Plate**
   One plate of 96 wells, Catalog No. 80-2385
   A clear plate of break-apart strips coated with a monoclonal antibody specific for IL-13

4. **Horseradish peroxidase IL-13 Antibody**
   10ml, Catalog No. 80-2384
   A yellow solution of HRP conjugated monoclonal antibody to IL-13

5. **Wash Buffer Concentrate**
   27 mL, Catalog No. 80-1286
   Tris buffered saline containing detergents

6. **TMB Substrate**
   10 mL, Catalog No. 80-0350
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide

7. **Stop Solution 2**
   10 mL, Catalog No. 80-0377
   A 1N solution of hydrochloric acid in water

8. **IL-13 Assay Layout Sheet**
   1 each, Catalog No. 30-0303

9. **Plate Sealer**
   3 each

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

Protect substrate from prolonged exposure to light.

Activity of conju-gate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

Stop solution is caustic. Keep tightly capped.

Storage

Reagents require separate storage conditions.

All components of this kit, except the Standard, are stable at 4°C until the kit's expiration date. The standard must be stored at or below -20°C upon receipt.

Materials Needed but Not Supplied

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable beakers
5. Graduated cylinders
6. A microplate shaker
7. Lint-free paper for blotting
8. Microplate reader capable of reading 450nm
9. Software (such as AssayBlaster™ catalog number ADI-28-0002) for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

Reagent Preparation

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Standards can be made up in either glass or plastic tubes.

The standard should be handled with care due to the known and unknown effects of the antigen.

1. **Wash Buffer**

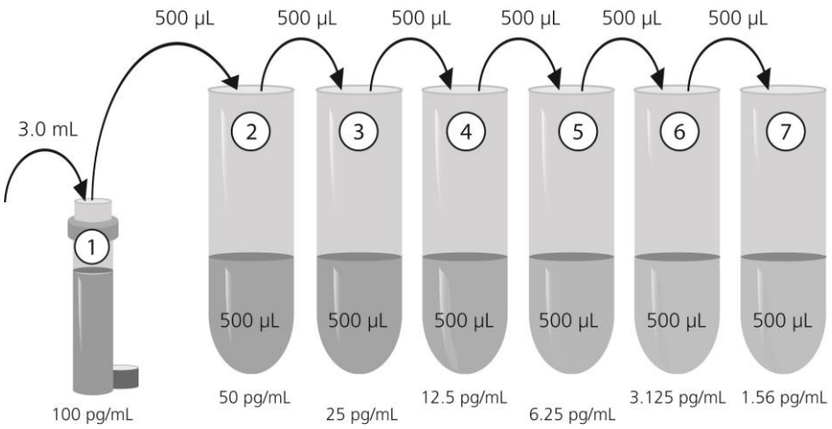
   Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

2. **Human IL-13 Standards**

   Allow the 300 pg human IL-13 standard to warm to room temperature. Reconstitute one vial of 300 pg human IL-13 Standard with 3.0ml standard diluent (Assay Buffer 13 or Tissue Culture Media) for a 100 pg/mL stock vial. Vortex thoroughly, wait 5 minutes and vortex again prior to use. Label this vial #1.

   Label six disposable 12 x 75 mm tubes #2 through #7. Pipet 500 µL standard diluent into each tube. Remove 500 µL from reconstituted stock vial and add to tube #2 and vortex thoroughly. Remove 500 µL from tube #2 and add to tube #3. Vortex thoroughly. Continue this for tubes #4 through #7.

   The concentration of human IL-13 in tubes #1 through #7 will be 100, 50, 25, 12.5, 6.25, 3.13 and 1.56pg/ml respectively (see image below). Standards should be used within 60 minutes of preparation.



Diluted standards should be used within 1 hour of preparation. The concentrations of the standards are labeled above.

Sample Handling

Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

The Enzo Life Sciences IL-13 (human) EIA kit is compatible with human IL-13 samples in culture supernates, plasma (with anticoagulant heparin or EDTA) and serum. Samples diluted sufficiently into the assay buffer can be read directly from a standard curve. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. A minimum 1:2 dilution is recommended for serum and plasma while cell culture supernates (with 10% serum supplement) may be run neat. These are the minimum recommended dilutions to remove matrix interference in the assay.

Samples in the majority of culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the culture media instead of Assay Buffer. Users should only use standard curves generated in media or buffer to calculate concentrations of human IL-13 in the appropriate matrix.

Dilutional Linearity

The minimum required dilution for several common samples was determined by serially diluting samples into the assay buffer and identifying the dilution at which linearity was observed. Conditioned RPMI with 10% fetal bovine serum (FBS) was spiked with recombinant human IL-13 and diluted in Assay Buffer. The assay buffer was spiked to the same concentration and used as a control to determine linearity of the culture medium. Multiple samples of human EDTA plasma and serum were diluted in the assay buffer to produce values within the dynamic range of the assay.

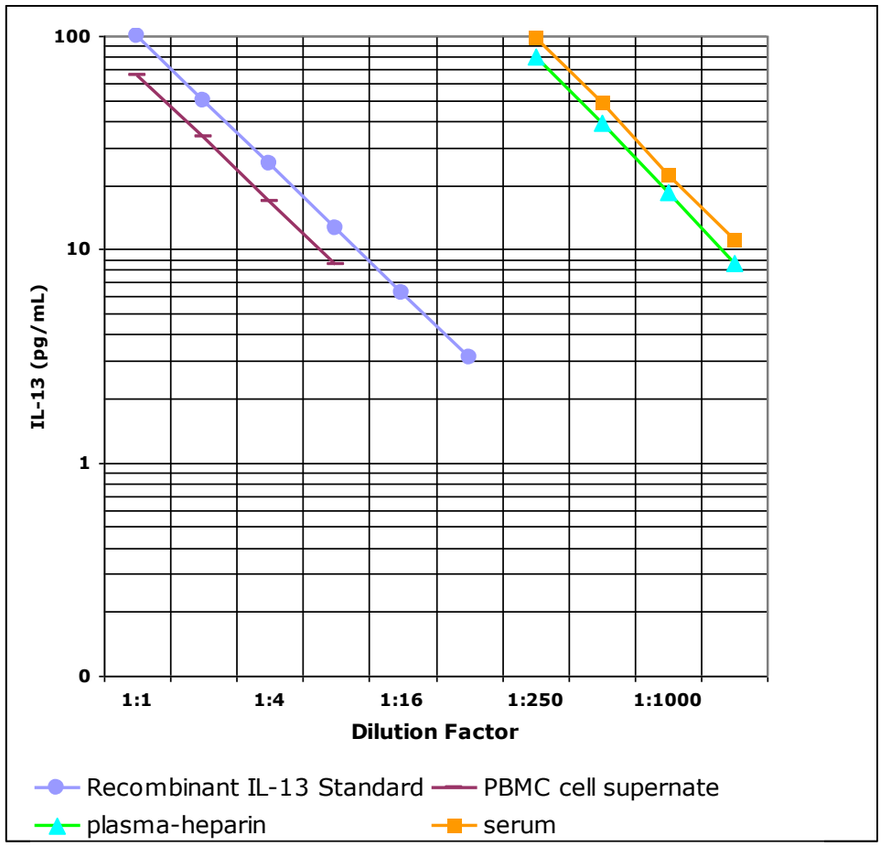
Matrix:	**Media**
MRD:	Neat
DF	**% Dil. Linearity**
Neat	93.8
1:2	96.6
1:4	98.7
1:8	92.0

Matrix:	**Plasma**
MRD:	1:2
DF	**% Dil. Linearity**
Neat	99.1
1:2	129
1:4	142
1:8	145

Matrix:	**Serum**
MRD:	1:2
DF	**% Dil. Linearity**
Neat	111
1:2	122
1:4	119
1:8	88

Parallelism

Parallelism experiments were carried out to determine if the recombinant human IL-13 standard accurately determines human IL-13 concentrations in biological matrices. Peripheral blood mononuclear cells (PBMCs) stimulated with the mitogen Phytohemaglutanin (PHA) were used to assess parallelism. Values were obtained using the cell supernatants from treated cultures serially diluted in assay buffer and assessed from a standard curve using four parameter logistic curve fitting. Human plasma and serum were similarly diluted in assay buffer and assessed again from a standard curve using four parameter logistic curve fitting. The observed values were plotted against the dilution factors. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples.



Spike and Recovery

After diluting each sample matrix to its minimum required dilution, recombinant human IL-13 was spiked at high, medium, and low concentrations. The recovery of the standard in spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean and range of percent recovery at the three concentrations are indicated below for each matrix.

Sample Matrix	Minimum Required	Spike Concentration	Recovery of Spike
Serum (n = 2)	1:2	80	96%
		20	90%
		5	96%
Human Heparin Plasma (n=4)	1:2	80	91%
		20	86%
		5	71%
TCM + 10% FBS (n=5)	neat	80	92%
		20	85%
		10	83%

Plasma Preparation

1. Collect whole blood in vacutainer tube containing anticoagulant (heparin or EDTA).
2. Centrifuge at 1000 x g for 15 minutes at 4˚C.
3. Place supernatant in a clean tube.
4. The supernatant may be divided into aliquots and stored at or below -20˚C, or used immediately in the assay.
5. Avoid repeated freeze-thaw cycles.

Serum Preparation

1. Collect whole blood in appropriate tube.
2. Incubate upright at room temperature for 30-45 minutes to allow clotting to occur.

3. Centrifuge at 1000 x g for 15 minutes at room temperature. Do not use brake.

3. Without disturbing the cell layer, place supernatant into a clean tube.

4. The supernatant may be divided into aliquots and stored at or below -20˚C, or used immediately in the assay.
5. Avoid repeated freeze-thaw cycles.

PBMC (Peripheral Blood Mononuclear Cells) Supernatant Preparation

1. Incubate PBMCs in RPMI supplemented with 10% FBS, L-glutamine and penicillin/streptomycin/amphotericin B for 48 hours.
2. Add phytohemaglutanin at 1 mg/mL. Incubate for 48 hours.
3. Collect cell supernatant.
4. Centrifuge at 1700 x g for 10 minutes at room temperature to pellet any cells or cellular debris.
5. Collect supernatant in a clean tube.
6. The supernatant may be divided into aliquots and stored at or below -20˚C, or used immediately in the assay.
7. Avoid repeated freeze-thaw cycles.

Assay Procedure

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the standards and samples to the bottom of the wells.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the foil pouch and seal the ziploc. Store unused wells at 4°C.

1. Pipet 100 µL of standard diluent (Assay Buffer 13 or Culture Media) into the S0 (0pg/ml standard) wells.
2. Pipet 100 µL of Standards #1 through #7 into the appropriate wells.
3. Pipet 100 µL of the Samples into the appropriate wells.
4. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
5. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100 µL of yellow Antibody Solution into each well, except the Blanks.
7. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
8. Wash as above (Step 5). Pipet 100 µL of Substrate Solution into each well.
9. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
10. Pipet 100 µL of Stop Solution to each well.
11. Zero the plate reader against the Blank wells and read the optical density (OD) at 450nm, preferably with correction between 570 and 590nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all of the readings.

Calculation of Results

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

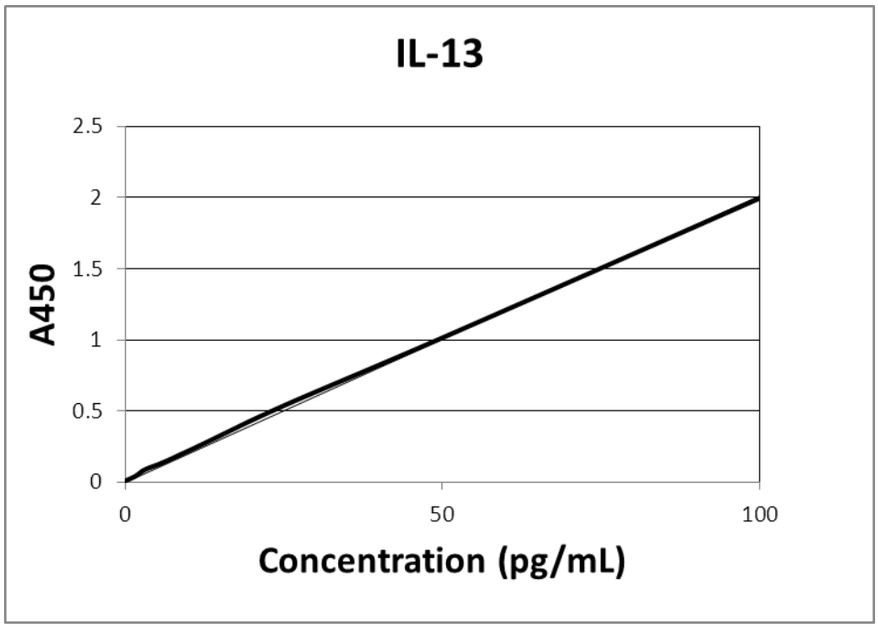
Several options are available for the calculation of the concentration of IL-13 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program (such as AssayBlaster™, catalog number ADI-28-0002). Such software is often supplied by plate reader manufacturers.

Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	IL-13(pg/mL)
S0	0.009	0
S1	2.0	100
S2	1.02	50
S3	0.54	25
S4	0.27	12.5
S5	0.14	6.25
S6	0.09	3.13
S7	0.04	1.56



Performance Characteristics

Specificity

The cross reactivities for a number of related compounds were determined by diluting cross reactants in the assay buffer at a concentration of 3,333-30,000 pg/mL. These samples were then measured in the assay.

Analyte	Cross Reactivity
IL-5	<0.01%
IL-15	<0.01%
IL-17α	<0.01%
IL-21	<0.01%
IL-29	<0.01%
IL-4	<0.01%
IL-8	<0.01%
IL-1β	<0.01%
IL-6	<0.01%
TNFα	<0.01%
RANTES	<0.01%
IFNγ	<0.01%
IL-1α	<1.00%
IL-2	<1.00%
IL-10	<1.00%
MCP-1	<1.00%
IL-13 mouse	<1.00%

Sensitivity

The sensitivity or limit of detection of the assay is 1.71pg/ml. The sensitivity was determined by interpolation at 2 standard deviations above the mean signal at back-ground (0pg/mL) using data from at least 6 standard curves.

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing IL-13 in a single assay.

pg/mL	%CV
51.5	6.15
12.4	2.89
2.8	7.46

Inter-assay precision was determined by measuring buffer controls of varying IL-13 concentrations in multiple assays over several days.

pg/mL	%CV
78.8	4.3
20.3	5.0
5.1	15.8

References

1. Kips, JC. Cytokines in Asthma. *European Respiratory Journal* 2001; 18: Suppl. 34, 24s-33s.
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Notes

Notes

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TRADEMARKS AND PATENTS

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